Comparison of Application Methods for Suppressing the Pecan Weevil (Coleoptera: Curculionidae) with *Beauveria bassiana* Under Field Conditions

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ABSTRACT The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is pathogenic to *C. caryae*. One approach to managing *C. caryae* may be application of *B. bassiana* directed toward adult weevils as they emerge from the soil to attack nuts in the tree canopy. Our objective was to compare different application methods for suppression of *C. caryae* adults. Treatments included direct application of *B. bassiana* (GHA strain) to soil under the tree canopy, soil application followed by cultivation, soil application in conjunction with a cover crop (Sudan grass), direct application to the tree trunk, and application to the trunk with an UV radiation–protecting adjuvant. The study was conducted in a pecan orchard in Byron, GA, in 2005 and 2006. Naturally emerging *C. caryae* adults, caught after crawling to the trunk, were transported to the laboratory to determine percentage mortality and signs of mycosis. When averaged over the 15-d sampling period, weevil mortality and signs of mycosis were greater in all treatments than in the nontreated control in 2005 and 2006; >75% average mortality was observed with the trunk application both years and in the trunk application with UV protection in 2005. Results indicated trunk applications can produce superior efficacy relative to ground application, particularly if the ground application is followed by cultivation. Efficacy in the cover crop treatment, however, did not differ from other application approaches. Future research should focus on elucidating the causes for treatment differences we observed and the extent to which *B. bassiana*–induced *C. caryae* mortality reduces crop damage.

KEY WORDS *Beauveria bassiana*, biological control, *Curculio caryae*, pecan

Pecan (*Carya illinoensis*) is an important nut crop in North America (Wood 2003). The pecan weevil, *Curculio caryae* (Horn), is a major pest of pecans throughout the southeastern United States, as well as portions of Texas and Oklahoma (Payne and Dutcher 1985). The insects have a 2- or 3-yr life cycle (Harris 1985). Adults emerge from soil in late July–August to feed on and oviposit in developing nuts (Harris 1985). Larval development is completed within the ripening kernel of the nut. The fourth instars drop to the soil and burrow to a depth of 8–25 cm, form a pupal cell, and overwinter. The following fall, ≈90% of the larvae pupate and spend the next 9 mo in the soil as adults (Harris 1985). The remaining 10% of the population spend 2 yr in the soil as larvae and emerge as adults in the third year (Harris 1985).

Current control recommendations for *C. caryae* consist mainly of aboveground applications of chemical insecticides (e.g., carbaryl) to suppress adults (Harris 1999, Hudson et al. 2006). Application of chemical insecticides is recommended every 7–10 d during peak *C. caryae* emergence (generally up to at least a 6-wk period) (Hudson et al. 2006). Because of problems associated with aphid and mite resurgence that often result from chemical applications (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic fungi are one of the potential alternatives (Shapiro-Ilan 2003).

The most studied entomopathogenic fungus for *C. caryae* control to date is *Beauveria bassiana* (Balsamo) Vuillemin (Gottwald and Tedders 1983, 1984, Sikorowski 1985, Harrison et al. 1993, Fuxa et al. 1998, Shapiro-Ilan et al. 2003, 2004). Hypocreales, such as *B. bassiana*, invade the insect host through the cuticle, replicate in the host hemocoel, and form external conidiophores to disperse their spores (Tanada and

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Kaya 1993). *B. bassiana* is pathogenic to a variety of insects including a number of curculionid and other coleopteran pests (Harrison et al. 1993, Tanada and Kaya 1993, Booth et al. 2000, McCoy et al. 2000, Shapiro-Ilan et al. 2003).

Laboratory studies have indicated relatively high virulence of some *B. bassiana* strains to *C. caryae* larvae (Harrison et al. 1993, Shapiro-Ilan et al. 2003). Field studies targeting *C. caryae* larvae, however, have resulted in highly variable levels of control ranging from <30 (Gottwald and Tedders 1983, Harrison et al. 1993) to 62% (Tedders et al. 1973). Additionally, a potential drawback to larval control with *B. bassiana* is that larvae emerge from nuts over several months, i.e., October to December (Boethel and Eikenbary 1979, Harris and Ring 1979), and thus a lack of fungal persistence (Shapiro-Ilan et al. 2004) could require multiple applications. In contrast, the bulk of *C. caryae* adults emerge from soil over a 4- to 6-wk period (Harris 1976), requiring fewer applications.

One approach to controlling *C. caryae* may be to expose the adult insects to *B. bassiana* when they are emerging, i.e., before they enter the canopy to feed and oviposit. Prior research has indicated that a high proportion (>90%) of emerging *C. caryae* either crawl or fly to the trunk (Raney and Eikenbary 1968). By exploiting this behavior, significant control might be achieved by applying *B. bassiana* to the trunk or to soil under the canopy where the insects are emerging.

Although the idea of targeting emerging *C. caryae* with entomopathogenic fungi has been addressed previously, research has yet to establish that sufficient efficacy can be achieved. Gottwald and Tedders (1983) showed promise in the approach; when *B. bassiana* was applied around the base of pecan trees and adult *C. caryae* were released within 15-cm-high enclosures near the point of inoculation, 78% (corrected with Abbott's formula, Abbott 1925) of the *C. caryae* that crawled to the trunk were killed by the fungus. Gottwald and Tedders (1983), however, only tested immediate effects on artificially released *C. caryae* and did not attempt to measure how long the effect of a perimeter *B. bassiana* application might persist. Shapiro-Ilan et al. (2004) measured the effect of *B. bassiana* ground applications made to soil in a 2-m radius around the tree trunk during a 9- to 16-d period. Although (similar to Gottwald and Tedders 1983) high levels of *C. caryae* mortality were observed shortly after application (e.g., up to 95% mortality within first 3 d), Shapiro-Ilan et al. (2004) did not detect any treatment effects after the first week after application, and average weevil mortality during the 16-d period was <65%. To ensure the economic viability of the approach, residual treatment effects comparable to (or longer than) chemical insecticides will be required.

We hypothesized that novel methods or conditions of application may improve *B. bassiana* suppression of *C. caryae* beyond what had been observed previously (e.g., by Shapiro-Ilan et al. 2004). Because of the high proportion of adult weevils that crawl or fly to the trunk, direct applications of *B. bassiana* to the trunk may facilitate infection. However, because some sunlight penetrates the canopy (Wood and Stahmann 2004, Lombardini 2006) and UV radiation is detrimental to *B. bassiana* (Goettel et al. 2000), we hypothesized that addition of a UV-protecting adjuvant could enhance fungal persistence and efficacy on the trunk. Similarly, we hypothesized that methods to protect the fungus after soil application such as tillage (Gaugler et al. 1989) or including a cover crop would be beneficial. Thus, our objective was to compare methods of *B. bassiana* application for adult *C. caryae* suppression; treatments included application directly to soil, soil application followed by cultivation, soil application with a cover crop, trunk application, and trunk application with a UV-protecting adjuvant.

Materials and Methods

Field experiments to compare different methods of *B. bassiana* application for adult *C. caryae* suppression were conducted in a pecan orchard at the USDA–ARS research farm in Byron, GA, in 2005 and 2006. The orchard consisted of mature pecan trees (mixed Stuart and Schley varieties) ≈100 yr old, with an average diameter of ≈2 m and spaced 20 m apart. Soil type was a loamy-sand (80:16:4, sand:silt:clay; pH = 6.1). The experiments were conducted in a randomized complete block design with four blocks (tree rows) of five treatments and an untreated control. Each plot consisted of a single tree (thus there were four rows of six trees in the experiment). The application rate for all *B. bassiana* treatments was 5×10^{12} conidia per tree.

Beauveria bassiana (GHA strain), i.e., Botanigard, which was used in all field experiments, was obtained from Emerald BioAgriculture (Butte, MT) as an emulsifiable oil formulation containing 2×10^{13} conidia per 946-ml container. This strain (GHA) has been labeled for use in controlling pecan weevil. The material was stored at ≈4°C and used within 2 mo of receipt. Before field application in 2005 and 2006, viability of conidia was verified according to percentage germination (on agar) as described by Goettel and Inglis (1997). Separately, before the 2005 field experiments, a test was conducted to determine if the adjuvants or carriers used in field applications (see below) affected *B. bassiana* germination. Specifically, germination of unformulated *B. bassiana* conidia was determined when suspended in sunflower oil (ConAgra Foods, Irving, CA) or SoyScreen (distilled at USDA–NCAUR, Peoria, IL), or as dry powder, and stored at 25°C for 1 or 28 d (the dry powder was suspended in water just before assessment). Germination was determined by microscopic observations for developing germ tubes (three counts per suspension) after a 14-h incubation at 25°C and 260 rpm in yeast extract broth (Behle 2006).

Treatments were applied on 15 August 2005 and 2006 and consisted of fungal application to soil surrounding the trunk, soil application followed by cultivation, soil application with a cover crop, trunk application, and trunk application with a UV-protecting adjuvant. All soil treatments were applied within a 5-m radius of the tree trunk (hence the rate per unit area was $\approx 6.4 \times 10^{10}$ conidia/m^2); *B. bassiana* for each tree was mixed with 30.3 liters of water and applied using water cans. In the cultivation treatment, the soil within the 5-m radius from trunk was tilled to a depth of $\approx$7.5 cm with a tractor-mounted disk before application and mixed using hand rakes to a depth of $\approx$5 cm 1 d after application. The cover crop, which was contained within the 5-m radius from the trunk, consisted of Sudan grass, *Sorghum bicolor* L. Moench, $\approx$20.3 cm in height; plots in other treatments were essentially void of ground cover. In 2005, the cover crop treatment was applied in only three plots (otherwise, all other treatments and controls were applied to four plots both years). For the trunk application treatment, *B. bassiana* (236.5 ml BotaniGard ES; i.e., 5×10^{12} conidia) was mixed with sunflower oil to reach a total volume of 1 liter and applied to $\approx$1.5 m of the bottom portion of the trunk using a CO_2 charged backpack sprayer (310.3 kPa; Spray Systems Co., Wheaton, IL) with a cone nozzle (5500-X8 adjustable conejet). The trunk treatment with UV-protecting adjuvant was identical except that 100 ml of the sunflower oil was replaced with 100 ml of SoyScreen oil. SoyScreen has been shown to provide UV protection (Compton and Laszlo 2002). Control plots received 30.3 liters of water applied to the soil as in the ground treatments.

Adult *C. caryae* were collected in Circle traps attached to pecan trunks (Mulder et al. 2003). This is a passive trap that captures weevils crawling up the trunk. The traps were made of wire mesh (1.5 mm mesh) with an open area ($\approx$61 cm wide) facing toward the soil (to collect ascending weevils) and tapering up to a removable top. Traps were placed on the trunk so that the bottom of the trap was $\approx$100 cm above the soil surface. The top of the trap (the removable one-way cone portion where weevils accumulate) was placed on the trap $\approx$24 h before each collection (sample date). Five traps were placed on each tree so that the entire circumference of the trunk was covered. *C. caryae* were collected in traps 1, 3, 8, 10, and 15 d after treatment. To avoid contamination among plots, we placed plastic bags over our shoes just before entering plots treated with *B. bassiana* and removed the bags on exiting. Daily average, maximum, and minimum temperatures for soil (5 cm below the surface) and air, as well as total precipitation, were recorded during the 15-d experimental period; these data were collected from a weather station located on the USDA–ARS research farm $\approx$0.64 km from the application site.

On each day that *C. caryae* were trapped in the field, the insects captured in each trap were placed in separate plastic bags and transported to the laboratory to determine levels of fungal infection. All *C. caryae* were placed individually in 30-ml plastic cups (3–4 cm ID, 3.5 cm deep) with a 3-cm cotton wick moistened with $\approx$2.1 ml of tap water. Cups were placed in plastic boxes (28 by 15 by 9.5 cm deep) organized by block and incubated in darkness at 25°C. After 14 d of incubation, the percentage *C. caryae* mycosis per plot was estimated by examining the cadavers for signs of fungal infection (Goettel and Inglis 1997, Shapiro-Ilan et al. 2004). The percentage of total *C. caryae* mortality (mycosis plus other causes) was also recorded.

Although the distance between plots was substantial ($\geq$20 m) (see Lacey et al. 2000), and thus most weevils captured were likely to have originated in the plot they were captured in, it is conceivable that some weevils could have entered a plot from neighboring plots, e.g., because of inter-tree dispersal (Raney et al. 1969). Thus, in 2005, we estimated the potential impact of weevil movement on insect captures and mortality assessment by placing a cone emergence trap (Polles and Payne 1972) on the soil surface in each untreated plot. Because the cone traps were placed directly on the soil surface, they could only capture insects emerging from within plots. Hence, the potential impact of weevil movement was estimated in the untreated plots by comparing insect mortality or mycosis from cone traps relative to captures in Circle traps. Cone traps were made of aluminum screening (0.03-cm mesh, and dimensions of 70.5 cm bottom diameter, fitted with boll weevil traps on top) (Boethel et al. 1976, Duncan et al. 2001). The traps were placed $\approx$2 m from the trunk on a random side of the tree. Weevils were collected from the cone traps 3, 8, 10, and 15 d after treatment and processed as described above.

Differences among treatments in mortality and mycosis were analyzed by averaging effects over the entire experimental period and (separately) on each sampling date, i.e., similar to the approach of McCoy et al. (2000) and Shapiro-Ilan et al. (2003, 2004). Treatment effects averaged over the 15-d sampling period were analyzed using repeated-measures analysis and LSMEANS (Proc Mixed; SAS Institute 2002). A separate repeated-measures analysis was conducted to compare weevil mortality and mycosis within untreated plots from cone traps versus Circle traps. Additionally, cumulative treatment effects were analyzed by day (for each sampling date separately) using analysis of variance (ANOVA) and Student–Newman–Keuls' test (SAS Institute 2002). Cumulative treatment effects were based on the percentage of weevils that died or showed mycosis relative to the total number captured up to that point in the experiment (e.g., cumulative effects on sampling date 10 included weevils captured on sampling dates 1, 3, 8, and 10). Note that for the purposes of reporting, e.g., in the results section below and in the figures, mortality and mycosis from "control" plots refers only to Circle trap captures (which were congruent in trapping method with the treatment plots); mortality and mycosis in the cone trap captures were kept separate and used primarily for assessment of insect movement as described above. All percentage data were transformed by arcsine of the square root before analysis (nontransformed means are presented in the Results section). The α level for all statistical tests was 0.05.

Table 1. Statistics from field experiments conducted in 2005 measuring *B. bassiana* suppression of *C. caryae* in a pecan orchard, Byron, GA

Test	*F*	df	*P*
Average mortality over 15-d sampling period	12.0	5,14	0.0001
Average mycosis over 15-d sampling period	11.53	5,14	0.0001
Cumulative mortality 3 DAT	8.49	6,13	0.0007
Cumulative mortality 8 DAT	11.84	6,16	0.0001
Cumulative mortality 10 DAT	5.43	6,16	0.0031
Cumulative mortality 15 DAT	4.44	6,16	0.0079
Cumulative mycosis 3 DAT	8.6	6,13	0.0006
Cumulative mycosis 8 DAT	6.35	6,16	0.0014
Cumulative mycosis 10 DAT	4.55	6,16	0.0071
Cumulative mycosis 15 DAT	8.31	6,16	0.0002

Results

For field experiments, viability counts (based on the agar plate technique) of the *B. bassiana* product in 2005 and 2006 indicated ≈83.5 and 80.6% germination, respectively. Germination percentages (average ± SD, based on the yeast extract broth technique) among carriers/adjuvants, SoyScreen, sunflower oil, and dry conidia were 93.3 ± 2.2, 89.0 ± 2.0, and 91.3 ± 0.9 after 1 d, respectively, and 86.7 ± 2.2, 91.3 ± 0.3, and 91.0 ± 1.5 after 28 d, respectively.

During the 15-d experimental period, average daily minimum and maximum ambient temperatures were 22.43 ± 1.42 and 33.26 ± 1.85°C (ranging from 20.0 to 35.56°C) in 2005 and 21.70 ± 1.19 and 33.09 ± 1.35°C (ranging from 18.89 to 35.56°C) in 2006, respectively. Average daily minimum and maximum soil temperatures at 5 cm below the surface were 26.42 ± 1.31 and 32.50 ± 1.42°C (ranging from 22.22 to 34.44°C) in 2005 and 26.01 ± 0.71 and 32.50 ± 0.91°C (ranging from 25.0 to 33.89°C) in 2006, respectively. Overall average daily ambient temperatures were 26.57 ± 1.35°C in 2005 and 26.34 ± 0.86°C in 2006, and overall average soil temperatures were 28.90 ± 0.93°C in 2005 and 28.92 ± 0.55°C in 2006. Total precipitation (during the 15-d period) was 65.0 mm in 2005 and 101.6 mm in 2006.

In 2005, when averaged over the 15-d sampling period, percentage *C. caryae* mortality and signs of mycosis were higher in all treatments than in the nontreated control (Table 1; Fig. 1). Average percentage (±SE) mortality reached 80.2 ± 7.3 (in the trunk plus SoyScreen treatment; Fig. 1A). Weevil mortality was higher in plots that received the trunk plus SoyScreen application than in the cultivation treatment (Fig. 1A). No other differences among *B. bassiana* treatments were detected in average mortality or mycosis in 2005 (Fig. 1). Additionally, when averaged over the experimental period, no difference was detected in mortality or mycosis in control plots between weevils captured in cone traps versus Circle traps ($F = 1.10$; df = 1,2; $P = 0.40$ for total mortality and $F = 0.08$; df = 1,2; $P = 0.80$ for mycosis). Average (±SE) mortality was 20.1 ± 12.9 and 31.0 ± 4.2 in the cone and Circle traps, respectively. Average mycosis was 13.9 ± 8.0% and 13.1 ± 3.8% in the cone and Circle traps, respectively. The total number of *C. caryae* captured in 2005 was 649, with an average of 129.8 ± 32.6 (SD) per sampling date.

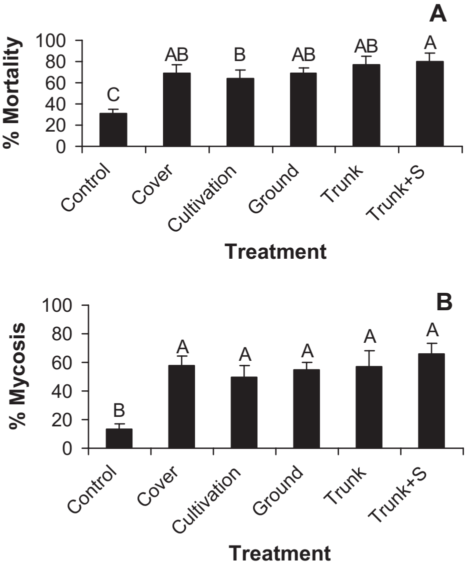

Fig. 1. Percentage adult *C. caryae* mortality (A) and weevils exhibiting signs of mycosis (B) after applications of *B. bassiana* in a pecan orchard in Byron, GA. Bars represent mean percentages (±SE) averaged over a 15-d sampling period in 2005. Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

When averaged over the 15-d sampling period, results in 2006 were similar to those observed in 2005. Higher mortality and mycosis were observed in all *B. bassiana* treatments (when averaged over the 15-d sampling period) than in the control (Table 2; Fig. 2). As in 2005, the only difference among *B. bassiana* treatments involved the cultivation treatment, but in 2006, mortality in the cultivation treatment was lower than the trunk application without SoyScreen (Fig. 2A). Average percentage mortality reached 78.3 ±

Table 2. Statistics from field experiments conducted in 2006 measuring *B. bassiana* suppression of *C. caryae* in a pecan orchard, Byron, GA

Test	*F*	df	*P*
Average mortality over 15-d sampling period	4.78	5,5	0.0082
Average mycosis over 15-d sampling period	4.64	5,5	0.0093
Cumulative mortality 3 DAT	3.62	5,15	0.0239
Cumulative mortality 8 DAT	1.33	5,15	0.3043
Cumulative mortality 10 DAT	8.00	5,15	0.0008
Cumulative mortality 15 DAT	7.55	5,15	0.0010
Cumulative mycosis 3 DAT	13.64	5,15	0.0001
Cumulative mycosis 8 DAT	1.39	5,15	0.2834
Cumulative mycosis 10 DAT	4.44	5,15	0.0111
Cumulative mycosis 15 DAT	7.59	5,15	0.0010

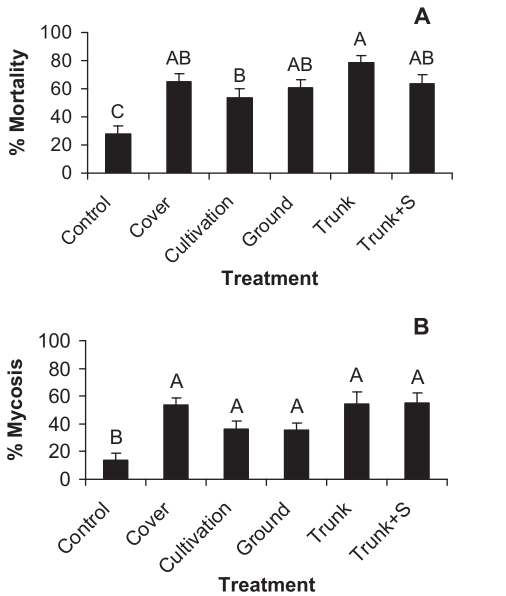

Fig. 2. Percentage adult *C. caryae* mortality (A) and weevils exhibiting signs of mycosis (B) after applications of *B. bassiana* in a pecan orchard in Byron, GA. Bars represent mean percentages (±SE) averaged over a 15-d sampling period in 2006. Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

5.1% (SE; in the trunk application; Fig. 2A). The total number of *C. caryae* captured in 2006 was 657, with an average of 131.3 ± 44.0 (SD) per sampling date.

In 2005, when cumulative mortality was analyzed on each sample date, higher percentages of *C. caryae* mortality were observed in all treatments compared with the control (Circle trap captures) 3 and 8 d after treatment but not 10 or 15 d after treatment (Table 1; Fig. 3). In contrast, percentage of weevils showing signs of mycosis was higher in *B. bassiana* treatments than the control (Circle trap captures) on all sampling dates (Table 1; Fig. 4). No differences in *C. caryae* mortality or mycosis among the *B. bassiana* application methods or between cone versus Circle trap captures were detected on any sample dates (Figs. 3 and 4). Because of low numbers of captured weevils that could be included in the cumulative mortality/mycosis analyses 1 d after treatment (ranging from 10 to 23 weevils per treatment except the cover crop, which had 30 weevils), this sampling date was omitted from these analyses. Cumulative mortality/mycosis analysis for all subsequent sample dates included a total of >30 weevils per treatment (with one exception, i.e., the trunk treatment 3 d posttreatment only had 13 weevils).

In 2006, when cumulative mortality was analyzed on each sample date, percentage *C. caryae* mortality was not different among treatments and the control 3 and 8 d after application, yet all treatments produced higher mortality than the control 10 and 15 d after application (Table 2; Fig. 5). Additionally, data from sampling dates 10 and 15 indicated higher mortality for

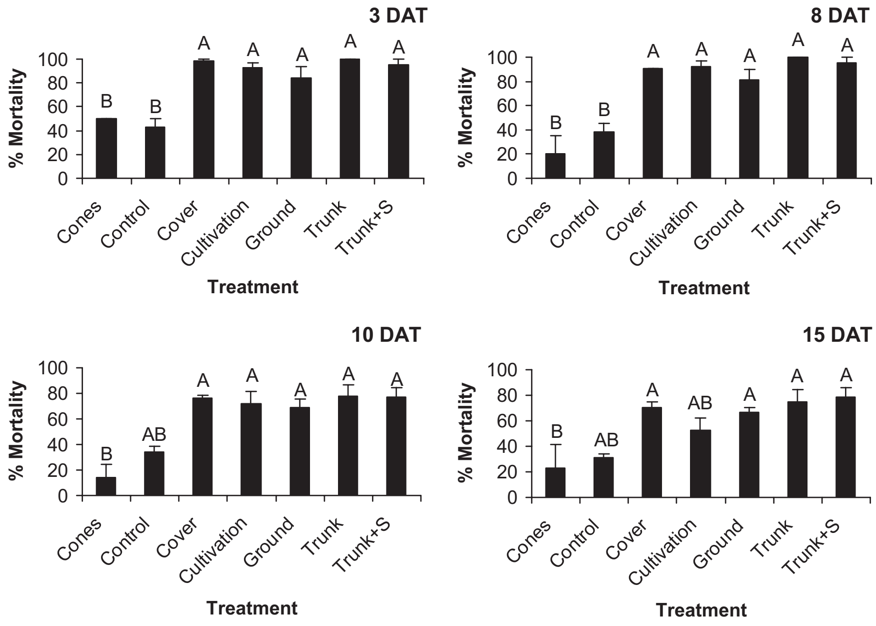

Fig. 3. Percentage adult *C. caryae* mortality after applications of *B. bassiana* in a pecan orchard in Byron, GA, 2005. Bars represent cumulative mean percentages (±SE) 3, 8, 10, and 15 d after treatment (DAT). Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

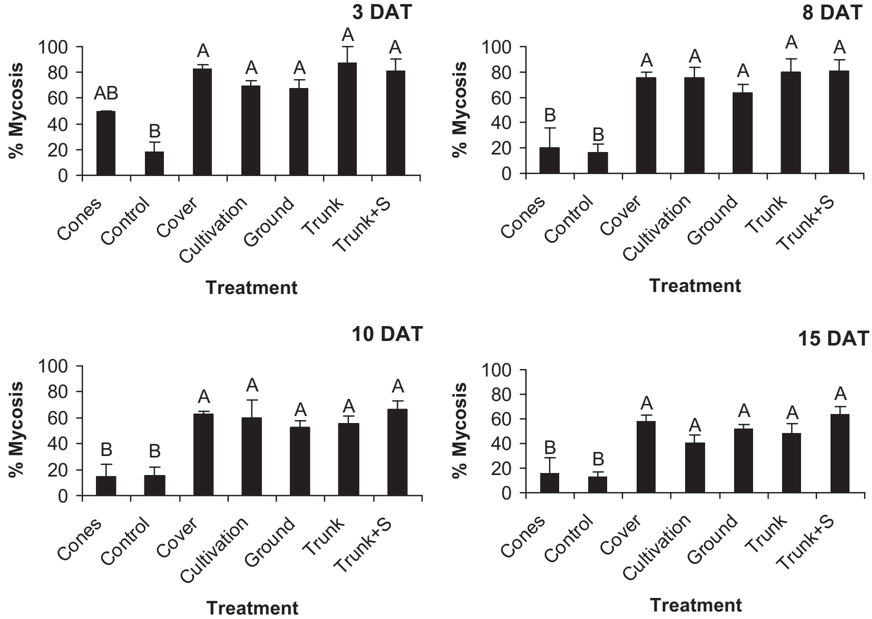

Fig. 4. Percentage adult *C. caryae* exhibiting signs of mycosis after applications of *B. bassiana* in a pecan orchard in Byron, GA, 2005. Bars represent cumulative mean percentages (±SE) 3, 8, 10, and 15 d after treatment (DAT). Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

the trunk application than the cultivation treatment (Fig. 5). The cumulative percentage of weevils exhibiting signs of mycosis was not different among treatments and the control on sampling date 8, but all treatments exhibited higher levels of mycosis 3 and 15 d after application, whereas only the trunk applications (with and without SoyScreen) exhibited higher mycosis than the control 10 d after application (Table 2; Fig. 6). Percentage mycosis in weevils captured 3 d after application was higher in the trunk and trunk plus SoyScreen treatment than the ground or cultivation treatment (which were not different from each other), and the cover crop treatment was intermediate (Fig. 6). Similar to the analysis of 2005 data, samples collected 1 d after treatment were omitted from the 2006 mortality/mycosis cumulative analysis because few weevils had been collected (ranging from 9 to 24). Cumulative mortality/mycosis analysis for all subsequent sample dates included a total of >30 weevils per treatment.

Discussion

All methods of *B. bassiana* application caused *C. caryae* mortality, and in many of the analyses (evaluated over the entire experimental period or on specific sample dates), differences among *B. bassiana* treatments were not detected. Nonetheless, there is evidence that trunk applications can cause higher mortality, or mycosis, than ground applications, particularly if the ground applications are followed by cultivation. The ground application that was applied to plots with a cover crop did not differ from other treatments in any of the analyses conducted. We expected the trunk application with SoyScreen to exhibit superior efficacy to that of trunk applications without a UV-protecting adjuvant, because of light penetration through the canopy; however, this hypothesis was not supported. The results suggest that either UV protection is not critical for efficacy when *B. bassiana* is applied to the trunk or that the SoyScreen protection did not transfer well under the field conditions tested.

A lack of (or reduced) exposure of weevils to microbial antagonists in trunk applications may explain the observed treatment differences between trunk and ground applications of *B. bassiana*. *B. bassiana* can be susceptible to degradation by microbial antagonists in soil (Lingg and Donaldson 1981, Fargues et al. 1983). Indeed, the fact that the cultivation treatment tended to produce the lowest *C. caryae* mortality supports the concept of microbial degradation as a causal factor because cultivation can enhance microbial activity (Paul and Clark 1989). Evidence of detrimental effects of tillage on efficacy or persistence on Hypocreales fungi, such as *B. bassiana,* has been observed in other studies (Sosa-Gomez and Moscardi 1994, Hummel et al. 2002). In contrast, Gaugler et al. (1989) observed enhanced persistence of *B. bassiana* in soil that was tilled versus untilled. A lack of persistence in ground applications of *B. bassiana* (GHA strain) is also reflected in the study by Shapiro-Ilan et al. (2004). However, some studies have indicated higher levels of persistence of *B. bassiana* GHA strain (Castrillo et al.

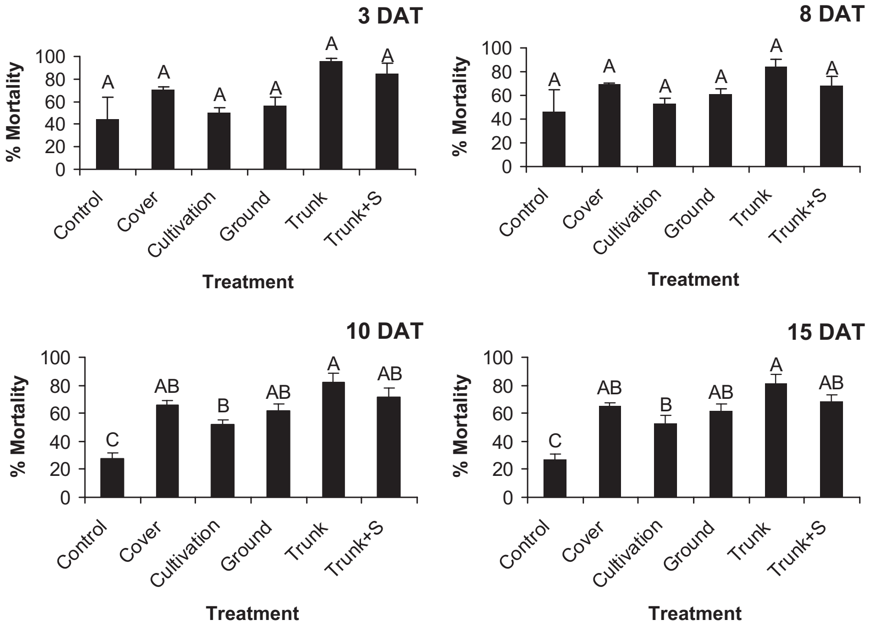

Fig. 5. Percentage adult *C. caryae* mortality after applications of *B. bassiana* in a pecan orchard in Byron, GA, 2006. Bars represent cumulative mean percentages (±SE) 3, 8, 10, and 15 d after treatment (DAT). Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

2003), other *B. bassiana* strains (Reay et al. 2007), or other Hypocreales (Bruck and Donahue 2007). The discrepancies in persistence are possibly caused by differing soil characteristics and associated microorganisms.

Alternatively, treatment differences may be caused by use of oil as a carrier in the trunk application versus water as a carrier in the soil treatments. Conceivably, the oil led to greater adherence or germination on crawling weevils relative to water. The oil was chosen because we expected superior adherence to the trunk (reduced run-off) relative to an aqueous spray, and choice of sunflower oil in particular was based on high levels of conidial viability after prolonged storage relative to other oils (R.W.B., unpublished data). We did not use oil in ground applications because adherence to the substrate was not an issue, and use of vegetable oils for ground application would not be economically feasible because of the volume required to treat the soil compared with the trunk; furthermore, water is the carrier specified on the manufacturer's label for ground treatments. We chose formulations that would have potential for use by growers. Because different carriers were used in the ground and trunk applications, we cannot determine whether the differences among treatments stem from the use of oil versus water or arise inherently from the differing application sites (soil versus trunk). Based on our germination tests, it seems that inherit differential persistence among the suspensions is not likely.

Another possible explanation for differences in treatment effects is concentration of conidia that weevils contacted. Although the total numbers of conidia applied to each plot was equal among treatments, the concentration of spores per unit area in the trunk applications was substantially higher than in the ground treatments. Furthermore, cultivating the soil reduced the density of conidia relative to applications that remained on the soil surface. Additional research is needed to determine why trunk applications caused higher mortality than ground treatments.

We hypothesized that a cover crop would provide protection to conidia (e.g., from UV radiation) and thus enhance efficacy. This hypothesis was supported in that, unlike the cultivation treatment and the direct (unamended) ground application, efficacy of the cover crop treatment was not found to be different from other fungal treatments on any occasion. Benefits to persistence or efficacy of Hypocreales through enhanced ground cover or shading have been studied previously (Sprenkel et al. 1979, Inglis et al. 1997, Hummel et al. 2002). For example, planting soybeans at higher densities (thereby decreasing light penetration to the soil) resulted in increased epizootics of *Nomuraea rileyi* (Farlow) (Sprenkel et al. 1979). Hummel et al. (2002) suggested the addition of a cover crop (red clover) may have contributed to increased Hypocreales prevalence; the use of the cover crop in this case, however, was not separated from other differing cultural practices such as tillage or application of biological versus chemical pesticides, and therefore specific effects of the cover crop could not necessarily be distinguished. Thus, to our knowledge, our study was the first to directly compare the effects of a cover

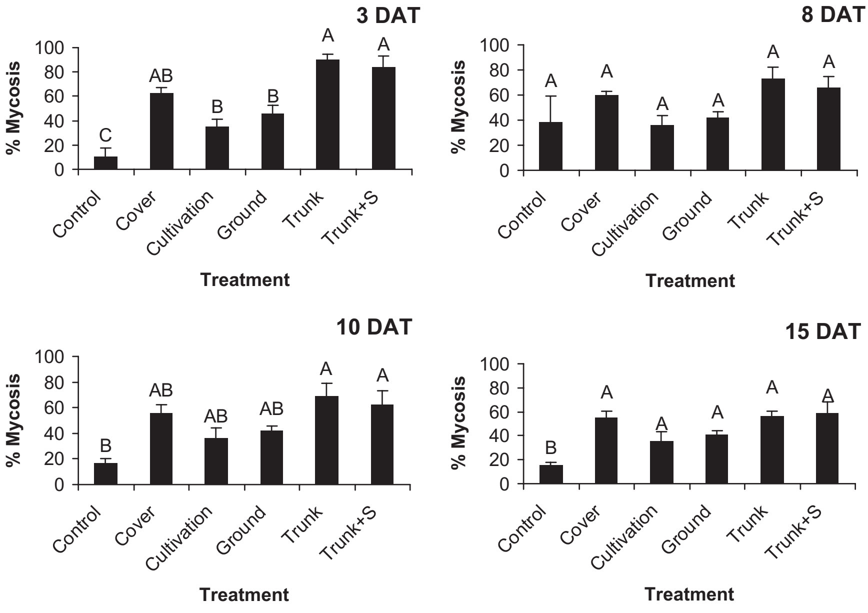

Fig. 6. Percentage adult *C. caryae* exhibiting signs of mycosis after applications of *B. bassiana* in a pecan orchard in Byron, GA, 2006. Bars represent cumulative mean percentages (±SE) 3, 8, 10, and 15 d after treatment (DAT). Approximately 5×10^{12} conidia were applied per tree. Fungal treatments were applied to the trunk with (Trunk + S) or without (Trunk) SoyScreen as a UV-protecting agent or to the ground with or without a cover crop or subsequent cultivation; a control received water only. Different letters above bars indicate statistically significant differences (Student–Newman–Keuls test, $\alpha = 0.05$).

crop on *B. bassiana* efficacy. Cover crops in combination with *B. bassiana* applications could be compatible with pecan management, e.g., a number of pecan systems already use forage under the canopy for cattle grazing (Wood 2003).

Another hypothesis for the observed cover crop effects is that the ground cover may have buffered temperatures (Hummel et al. 2002), thereby improving efficacy compared with other ground treatments. Indeed, the maximum temperatures recorded both in the soil and more so in the air reached levels that may be detrimental to *B. bassiana* GHA strain activity (Leland 2005, Leland et al. 2005). However, on average, the temperatures were within the range of fungal activity (20–30°C) (Goettel et al. 2000). Furthermore, it is unlikely that temperature extremes were the overriding factor in determining efficacy, because if temperatures were so important, one would expect the trunk applications (which were exposed to ambient temperatures) to have been the most severely affected, but in fact, fungal infection was equal or greater in trunk treatments compared with all ground treatments. Additionally, it must be noted that temperatures within the shaded plots were likely to have been less extreme than those recorded in the open weather station. Unfortunately, we did not measure soil temperatures in the plots with cover crop versus without, and thus have no direct evidence supporting or refuting the hypothesis.

It is conceivable that some horizontal transfer of *B. bassiana* took place within the traps. Specifically, it is possible that some of the weevils that picked up conidia before entering traps transferred spores to other weevils that entered the Circle traps. Thus, perhaps in some cases, estimates of mortality and mycosis may have been elevated relative to if the weevils had been held separately from the time of capture. However, the degree or likelihood of transfer might be considered minimal because of the short duration the weevils were held together (a maximum of 24 h). Also, it should be noted that some level of horizontal transfer from weevil to weevil also may occur in nature. Gottwald and Tedders (1983) showed that *B. bassiana* can be transferred from an infected *C. caryae* to healthy individuals, and this can conceivably occur in the tree canopy as weevils make contact, e.g., through mating. Therefore, to some extent, the level of horizontal transfer in our traps might have mimicked some of the transfer that occurs in nature.

The *B. bassiana* application methods used in this study, particularly in the trunk and cover crop treatments, may have potential for incorporation into a *C. caryae* management plan. We observed higher levels of control than prior field studies that applied *B. bassiana* for larval *C. caryae* control (Gottwald and Tedders 1983, Harrison et al. 1993). Additionally, we observed higher adult *C. caryae* mortality than an earlier study by Shapiro-Ilan et al. (2004), in which the maximum average mortality over a 16-d period was <65%, and no treatment effects were detected after 7 d after application. The ground applications made by Shapiro-Ilan et al. (2004) differed from those in this study in that the earlier study applications were made to a more narrow band (2 m) around the tree, which may have reduced exposure to the weevils, i.e., weevils flying to the trunk from outside the 2-m area would not have been affected by the treatment. Additionally, the formulation used in the earlier study was different (wettable powder as opposed to the ES formulation used in this study).

The high levels of *C. caryae* mortality observed in this study, however, may not necessarily translate into economic levels of crop protection. Because it can take ≥7 d for *B. bassiana* to kill *C. caryae* (Shapiro-Ilan et al. 2004), the weevils are likely to cause at least some feeding or oviposition damage to the nuts before the fungus takes effect. Feeding damage by weevils can occur essentially on emergence from the soil, whereas oviposition begins an average of 6.5 d after emergence and can continue for several weeks thereafter (Criswell et al. 1975). Furthermore, our evaluation is only an estimation of potential (as opposed to actual) field suppression of *C. caryae* by *B. bassiana.* This is because our analysis was based on *C. caryae* mortality after transport to controlled environmental conditions; thus, we cannot know how many of those weevils might have survived if they had remained in the field. Additional research is required to elucidate the causes for observed treatment differences and determine the potential of *B. bassiana* applications for protection of pecan crops.

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